Renewable and Sustainable Energy Reviews 26 (2013) 216–223

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

journal homepage: www.elsevier.com/locate/rser

Biodiesel production from heterotrophic microalgae through transesterification and nanotechnology application in the production

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ARTICLE INFO

Article history:
Received 23 October 2011
Received in revised form
22 May 2013
Accepted 26 May 2013
Available online 19 June 2013

Keywords:
Heterotrophic microalgae
Microbial oil
Transesterification
Biodiesel
Nanotechnology

ABSTRACT

Vegetable oils and animal fats are the most often used feedstock in biodiesel production; however, they are also used in food production, which results in increasing the feedstock price due to the competition. Therefore, alternative feedstock is required in biodiesel production. Heterotrophic microalgae are found capable of accumulating high lipid (up to 57% w/w). They can use complex carbons such as sweet sorghum and Jerusalem artichoke as nutrients to produce equivalent quantity oil as that of using glucose, which provides a cheap biodiesel production strategy. It was found that nanomaterials could stimulate microorganism metabolism, which suggested that nanomaterial addition in the cultivation could enhance lipid production of microalgae. Furthermore, the use of nanomaterials could improve the efficiency of the lipid extraction and even accomplish it without harming the microalgae. Nanomaterials such as CaO and MgO nanoparticles have been used as biocatalyst carriers or as heterogeneous catalyst in oil transesterification to biodiesel. In this paper, the factors that could impact on lipid accumulation of heterotrophic microalgae are critically reviewed; the advances on application of nanotechnology in microalgae lipid accumulation, extraction, and transesterification are addressed.

Contents

1. Introduction

At present, transesterification using plant oils, animal fats, or lipids from oleaginous microalgae is the major method of biodiesel production [1–3]. Among all the feedstock, oleaginous microalgae have gained a growing interest because of that conventional feedstock, plant oils which at present are the main source of biodiesel production, is becoming more and more unsustainable due to the strong competition with food production and kitchen utilization. The faster growth rate and greater lipid content of microalgae compared to oilseed crops urge researchers to develop the microalgae utilization in biodiesel production instead of plant oils [2,4]. In addition, the possibility of increasing lipid content of microalgae by controlling their cultivation condition, while which

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1364-0321/$ - see front matter
http://dx.doi.org/10.1016/j.rser.2013.05.061

is not possible for plant, offers another significant advantage [5]. Rodolfi et al. [6] selected four among 30 strains of microalgae to investigate the impact of cultivation condition such as irradiation and nutrient on lipid accumulation of the microalgae and found that lipid content significantly varied with the change of cultivation conditions.

Numerous studies have been reported on autotrophic microalgae used for production of biodiesel [7,8]. Autotrophic microalgae are capable of using carbon dioxide and solar energy to synthesize organics such as protein and lipid for their growth. Most of the production of autotrophic microalgae for biodiesel production occurs in indoor photobioreactors. The heavily light-dependent growth characterization of autotrophic microalgae resulting in energy consuming for illumination, as well as the low efficiency in the biomass productivity, has hindered autotrophic microalgae application in biodiesel production. In comparison, heterotrophic microalgae are more flexible for the cultivation condition (can grow under light free condition), and was found capable of accumulating higher lipid in the cells [9–11]. Miao and Wu [9] reported that the lipid content of heterotrophic *Chlorella protothecoides* was 3 times higher than that of the autotrophic one. Up to now, *Chlorella protothecoides* is the most studied heterotrophic algae as lipid source for biodiesel production [12–14].

Nanotechnology is the technique to devise, synthesize, manufacture and apply the matters with atomic or molecular precision at dimensions of 100 nm (nm) or smaller [15]. Nanomaterials have the surface area several hundred times more than their equal weight of macroscale materials. Not only is the surface area extensively increased, the tenacity, elasticity, strength and electricity are also enhanced.

There are many research fields and several potential applications that involve nanotechnology due to its unique behaviors and properties. Nanotechnology application in biodiesel production from microalgae mainly includes nanomaterial utilization on lipid accumulation, extraction and on the transesterification process as catalyst support or catalyst as shown in Fig. 1 [16–19]. In tradition, organic solvents having great affinity to lipid such as chloroform, hexane, isopropanol, and methanol are utilized in lipid extraction from microalgae; however, the use of toxic material (solvents), the difficulty of the complete recovery of the material, and the demand of the energy intense solvent–lipid separation step requires the development on extraction technology. The mechanism of solvent extraction is that solvent can weaken/break cell wall, and thus enhance lipid diffusion to the outer environmental/dissolve the lipid. Nanomaterials are favorable carriers in immobilization due to the high surface area, and solid nanomaterials can be easily recovered from liquid phase by filtration or centrifugation. Therefore, immobilizing organic solvent-like chemicals onto solid nanomaterials would solve the problems in organic solvent extraction. The immobilized chemicals as function group achieve the lipid extraction and were recovered as nanomaterials were recovered. A research revealed that modified nanosphere silica accomplished the extraction from alive microalgae which would be sent back for lipid accumulation again, and hence the process avoids recultivation [16]. It would be the immobilization of chemicals which weaken the cell wall (but not to kill the microalgae) and would thus lead to lipid diffusion from inside to outside of the cells.

The most employed catalyst in transesterification is acid or base; however, the corrosion (aggressive acid utilization) and soap formation (free fatty acid reacts with base) need alternative catalysts. Lipase, a biocatalyst, is environmentally friendly and efficient, but rather expensive, while the cost can be reduced when lipase immobilization is applied because of the possibility of lipase reuse. Nanomaterials have large surface area for immobilization and can be easily separated from products, hence, immobilizing lipase onto nanomaterials would benefit reducing the cost of using lipase [20]. Apart from being carriers, nanomaterials such as CaO, Al_2O_3, and MgO nanoparticles themselves are heterogeneous catalysts and can achieve high conversion rate (> 99%) with less addition amount (< 1% of oil addition) [21,22]. It is contributed by its high surface area which increased contacting chances with the reagent lipid. Moreover, comparing with the bulk materials, activity, lifetime, and resistance to poisons of their nanomaterials are improved [23,24]. Therefore, it suggested that nanomaterials catalysts could have high performance in transesterification.

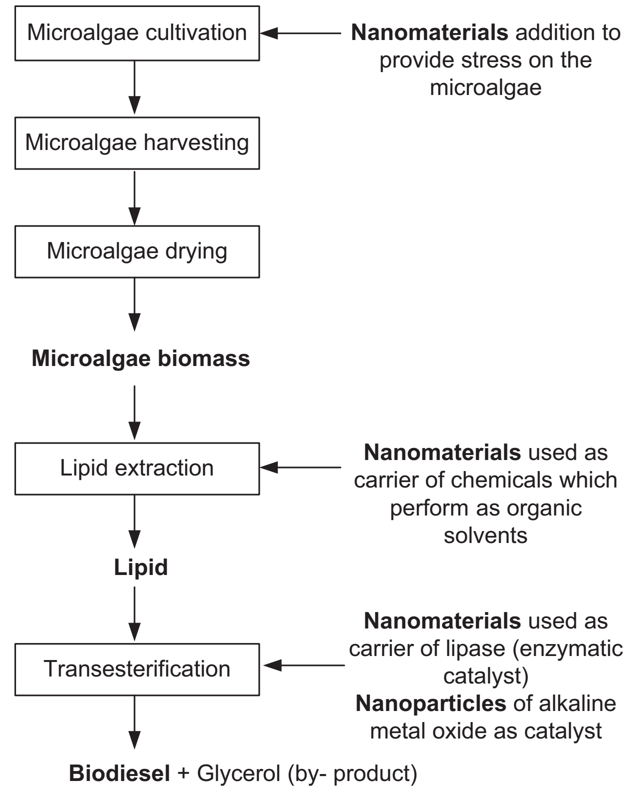


Fig. 1. Nanotechnology application in biodiesel production from heterotrophic microalgae.

Biodiesel production from heterotrophic microalgae includes microalgae lipid production (also called feedstock production) and the lipid transesterification to biodiesel. The lipid production including lipid accumulation and extraction is essential step as feedstock takes up to 70% of the overall cost [25,26]. Researchers have reviewed the methods including cultivation temperature, pH, the presence of radiation, and nutrient limitation, for enhancing lipid accumulation in microalgae [27]. Carbon and nitrogen source, carbon to nitrogen ratio, mineral presence, and the nanomaterial effect on the lipid accumulation have not been well addressed. The review on organic solvent extraction or pre-treatment (sonication, homogenization, microwave, bead milling) followed by solvent extraction for lipid extraction from microalgae have been reported [28,29]. Nanomaterial application on the extraction has not been discussed. Transesterification of lipid to biodiesel with various catalysts, the homogeneous and the heterogeneous, has been well analyzed [30,31]. Nanomaterial as a promising catalyst in transesterification should be paid significant attention. In this paper, the factors that could impact on lipid accumulation of heterotrophic microalgae are critically reviewed, and the advances of nanomaterial's application in lipid production are addressed. Additionally, the potential application of nanomaterials in biodiesel synthesis (transesterification) is proposed as well.

2. Lipid production from heterotrophic microalgae and nanomaterial application in the production

Lipid production from heterotrophic microalgae mainly includes the cultivation and the lipid extraction process. It is known that lipid production takes a major part of the overall cost of biodiesel production. Therefore, researchers and engineers have been working on increasing lipid content in microalgae by manipulating the cultivation conditions and improving lipid extraction efficiency by controlling the extraction steps [10,32,33].

2.1. Factors affecting lipid accumulation

Lipid content is the key factor of biodiesel production from heterotrophic microalgae. Strategies such as selection of carbon source and nitrogen source for enhancing lipid accumulation in microalgae have been reported [10–12,33]. Glucose is the most often used carbon source in heterotrophic microalgae cultivation; however, its high cost requires replacement which is cheaper and at least equally efficient [33]. Cheng et al. [12] investigated the effect of sucrose and sugar cane juice as carbon source on lipid production of heterotrophic microalgae, *Chlorella protothecoides*. It was found that lipid content was only slightly affected by the carbon source (Table 1). It indicates that sugar cane could be a suitable substitute of carbon source for producing heterotrophic microalgae oil. In addition, more complicated carbon sources such as Jerusalem artichoke and corn powder have been investigated for lipid accumulation of microalgae [14,33,34]. Cheng et al. [33] found that the lipid content of the microalgae *Chlorella protothecoides* cultivated with Jerusalem artichoke (44%), known as tuberous plant rich in carbohydrates, had almost no difference with that using glucose (45.2%) as carbon source. Xu et al. [14] obtained higher cell concentration and higher lipid content fed with corn powder (3.92 g/L and 55.3%), than those with glucose (3.74 g/L and 54.7%), respectively, at 144 h cultivation. Sweet sorghum is a well-known plant producing sugar-rich stems, of which the sugar is mainly sucrose (55% w/w) and cellulose (22.6% w/w) [35]. It was found that lipid accumulation content and yield in *Chlorella protothecoides* cultivated using sweet sorghum juice or glucose as carbon source showed no remarkable difference (52.7% w/w, 0.54 g/L/day for sweet sorghum juice and 53.3% w/w, 0.39 g/L/day for glucose) [34]. Complex carbon sources have shown good results in lipid accumulation of microalgae, which implies that it is feasible to use these cheap carbon sources for microalgae oil production. Shen et al. [11] studied the influence of nitrogen source (urea, yeast extract, and nitrate) on lipid productivity of heterotrophically cultivated *Chlorella protothecoides*. It was observed that the lipid yield in microalgae varied from a hundred to several hundred milligrams per liter per day according to the difference of the nitrogen source, and the highest yield (654 mg/L/day) was gave with nitrate. It suggests that nitrogen type significantly affects the lipid accumulation (lipid content varies from 25% to 46% w/w with different types of nitrogen source), which could be due to the

Table 1
Lipid accumulation in microorganisms.

Microbe	Lipid Content (% w/w)	Carbon source z(g/L)	Nitrogen source	C/N ratio	pH	Cultivation conditions			Reference
						Temp. (°C)	Shaking rate (rpm)	Period (h)	
Chlorella zofingiensis	52	Glucose (50)	Nitrate	143	6.5	25	150	144	[87]
	22	Lactose (50)	Nitrate	150	6.5	25	150	144	
	25	Galactose	Nitrate	143	6.5	25	150	144	
	51	Fructose	Nitrate	143	6.5	25	150	144	
	51	Sucrose	Nitrate	150	6.5	25	150	144	
	50	Mannose	Nitrate	143	6.5	25	150	144	
Chlorella protothecoids	55.2	Glucose (10)	Glycine	214	6.5	28	180	240	[14]
	54.7	Glucose (5)	Glycine	107	6.0	28	180	240	
	55.3	Corn powder (5)	Glycine	—	6.0	28	180	240	
Chlorella protothecoids	57.9	Glucose (10)	Glycine	214	6.5	25	200	240	[9]
Chlorella protothecoids	46.7	Glucose (20)	Yeast extract	31	6.3	28	200	108	[12]
	44.4	Sugar cane (20)	Yeast extract	12.5	6.3	28	200	108	
	53	Sugar cane (16.8)	Yeast extract	21	6.3	28	200	108	
	49	Sugar cane (16.8)	Yeast extract	15	6.3	28	200	108	
	42	Sugar cane (16.8)	Yeast extract	9	6.3	28	200	108	
Chlorella protothecoids	*50.5*	Glucose (40)	Nitrate	22.86	6.8	28	250	216	[11]
	27.3	Glucose (40)	Urea	19.8	6.8	28	250	216	
	33.4	Glucose (40)	Yeast extract	44.8	6.8	28	250	216	
Chlorella protothecoids	*52.5*	Sweet sorghum juice (10)	Yeast extract	16.7	—	28	220	120	[34]
	53.3	Glucose (10)	Yeast extract	11.8	—	28	220	120	
Chlorella protothecoids	*44*	Jerusalem Artichoke tuber (30)	Yeast extract	Yeast extract (4 g/L)	6	28	200	96	[33]
	45.2	Glucose (30)	Yeast extract	35.3	6	28	200	96	
Chlorella protothecoids	*44.3*	Glucose (10)	Yeast extract	21	6.5	28	200	200	[13]
Chlorella protothecoids	*57.8*	Glucose (15-60)	Yeast extract	17.6—70.4	6.5	28	200	184	[89]
Chlorella protothecoids	*23.5*	Glycerol (30)	Yeast extract	34.4	6.8	28	200	144	[90]
Schizochytrium limacinum	*51*	Glycerol (90)+ corn steep solid (10)	0	—	8	—	—	—	[91]
Schizochytrium limacinum	*18*	Glycerol (106)	0	—	7.5	20	170	—	[92]
Schizochytrium limacinum	*50.57*	Glycerol (75)	Nitrate+ammonia nitrogen	2000	7.5—8	20	170	—	[93]

impact of composition of nitrogen source on metabolic pathway of microalgae. Carbon to nitrogen (C/N) ratio has also been studied to optimize lipid accumulation of microalgae [12,13,33]. It was shown that higher C/N ratio led to higher lipid accumulation (Table 1). Nitrogen is an important nutrient in cell growth and division of microalgae. The size and number of cells in microalgae would increase under appropriate ratio of carbon and nitrogen supply. However, when carbon is sufficient but nitrogen is deprived, the cell division would be forced to cease and cell size growth would take place. The deprivation of nitrogen would inhibit the protein formation in the cell and thus result in lipid accumulation in the cells. Rodolfi et al. [6] reported that microalgae cultivated in nitrogen deficient condition (50% w/w lipid content) had given 18% w/w more lipid content than the one cultivated in nitrogen sufficient condition (32% w/w lipid content) with other conditions the same. Widjaja et al. [5] stated the similar result. Though heterotrophic microalgae have shown great capacity of lipid accumulation, the related study is rather limited. Except the impact of carbon source and nitrogen source on lipid accumulation, no other factors have been investigated till date. It was reported that environmental stress such as silica deprivation, pH, temperature, significantly affected lipid accumulation in autotrophic microalgae and fungi [5,6,36–38]; therefore, the factor can also impact on lipid accumulation of heterotrophic microalgae. Impropriate pH could inhibit microorganism activities and hence affects lipid production. The temperature effect on lipid accumulation is probably due to the self-protection that microorganisms accumulate lipid, which is major energy supplier for the living beings, for maintaining the normal life activities under the low temperature. Mineral concentration in culture medium could also affect lipid accumulation of heterotrophic microalgae. Some researchers have indicated that iron is an important substance in metabolism of living beings [39,40]. Menzyanova et al. [41] studied the iron effect on growth rate, protein content, and lipid content of autotrophic microalgae, *Dunaliella viridis Teod*., and reported that iron concentration in cultural medium showed impact on lipid content of the microalgae. It can be predicated that lipid content in heterotrophic microalgae could possibly be manipulated by adjusting iron concentration of the medium. Additionally, other minerals such as calcium and magnesium had also impact on lipid accumulation [42]. Moreover, periodic carbon depletion could also lead to variation of lipid content of the heterotrophic microalgae as it may adjust the metabolic pathway of lipid.

2.2. The effect of nanomaterial addition on lipid accumulation

Nanomaterials are found capable of enhancing microbe activities [43,44], and hence, it could be speculated that the addition of nanomaterials to microalgae cultivation medium could impact on lipid accumulation. It has been revealed that stress in cultivation such as low temperature (less than 20 °C), nutrient (nitrogen) depletion, high metal concentration (Fe), etc. triggered lipid accumulation [45,46]. The addition of nanoparticle such as silica or iron oxide nanoparticle in the medium causes strong sheer between cell and the nanoparticle which would be considered as competitor of nutrients by the cell. It threatens the cell to rapidly uptake nutrients and result in lipid accumulation.

Enhancing growth rate of heterotrophic microalgae would be an alternative for enhancing lipid productivity. Gao et al. [34] have proved that high growth rate could result in high lipid yield. It was reported that nanomaterials such as metal oxide nanoparticles (AgI/TiO_2) and single-walled carbon nanotubes were toxic to microbes [47,48]. However, Jin et al. [49] did not observe toxicity of nanomaterials on living cells. Williams et al. [50] investigated nanoparticle (silica, silica/iron oxide, gold) effects on growth and activity of *Escherichia coli* and reported that the addition of nanoparticles had no negative impact on growth and activity of *E. coli*. These studies indicated that appropriate selection of nanomaterials could possibly assist heterotrophic microalgae growth.

Lipid content directly affects the biodiesel production cost; therefore, the factors affecting lipid accumulation of heterotrophic microalgae should be further studied. Nanomaterial application in microalgae cultivation has great potential to increase lipid content and should be studied in future.

2.3. Nanomaterial application on lipid extraction

Lipid extraction is one of the major fractions of the cost of biodiesel production from microalgae. At present, the most common used method is solvent extraction in which organic solvents such as hexane, chloroform, methanol, or the combinations of the solvents, were used to reiteratively wash the wet or dried biomass of microalgae to obtain lipid [12,14,51]. It was displayed that the extraction yield of lipids varied largely while using different solvents [52]. The result showed that the lipid yield using chloroform and methanol was 20% w/w but it was only 15% w/w using hexane in the extraction. It suggests that the lipid extraction method is rather important in lipid production from microalgae, and should be paid attention for high yield. In addition to the selection of the solvents, the extraction condition could be also considered to improve lipid extraction yield. The utilization of irradiation and ultrasonication in extraction could assist the lipid yield [53]. However, these methods require additional energy consumption which may increase the extraction cost compared to solo solvent extraction. Recently, use of nanomaterials to enhance the extraction from microalgae has been introduced. Lu [18] reported that a type of nanomaterial had been synthesized and would be used in the extraction process from microalgae. It was predicated that this would be a favorable approach for lipid extraction because using nanomaterial could prevent the use of toxic materials (organic solvents) and the demand of complex solvent–lipid separation step in conversional extraction process. Furthermore, it has been reported that use of sphere nanomaterial to extract the lipid from living microalgae with no impact on microalgae lives which would be continuously used for lipid accumulation [16]. It indicates that nanomaterial instead of solvent, which kills the microalgae, lipid extraction would reduce the cost which is required for microalgae re-cultivation in solvent extraction case

3. Nanomaterial application on transesterification

Transesterification is the most applied technology in biodiesel production. It is a process using oils derived from animal, plant, or oleaginous microorganisms to react with alcohol (mainly methanol) for synthesizing fatty acid methyl esters—FAMEs (biodiesel). The reaction occurs either under extreme condition of high temperature and pressure or under mild condition in the presence of catalyst. Currently, biodiesel is mainly synthesized through the catalytic method. There are four types of catalyst, acid, base, enzyme, and heterogeneous catalysts, which have been studied in the synthesis. Biodiesel synthesis through transesterification is shown in Eq. (1).

$$\begin{matrix} CH_2\text{-}O\text{-}COR_1 \\ | \\ CH\text{-}O\text{-}COR_2 \\ | \\ CH_2\text{-}O\text{-}COR_3 \end{matrix} + 3\,EtOH \underset{}{\overset{Catalyst}{\rightleftharpoons}} \begin{matrix} CH_2\text{-}OH \\ | \\ CH\text{-}OH \\ | \\ CH_2\text{-}OH \end{matrix} + CH_3\text{-}O\text{-}COR_1 + CH_3\text{-}O\text{-}COR_2 + CH_3\text{-}O\text{-}COR_3 \quad (1)$$

where R_1, R_2, and R_3 are fatty acid chains; $CH_3–O–COR_1$, $CH_3–O–COR_2$, and $CH_3–O–COR_3$ are alkyl (methyl) esters.

Acids such as H_2SO_4 and HCl are usually used as catalysts in the reaction in lab scale studies [14,52], while bases such as NaOH and KOH are usually employed in industrial biodiesel production [54]. However, studies have pointed out that the acid catalyzed process needs extra care in reactor due to the aggressive characteristic of employed acid, and additionally, it usually requires excess methanol (molar ratio of methanol to oil is around 60:1) [52]. While base catalyzed biodiesel production consumes base due to the soap formation [55]. Compared to acid and base catalyzed processes, transesterification catalyzed by biocatalyst lipase is more environmentally friendly and efficient [56,57]; however, the use of costly raw material for lipase production has inhibited enzymatic biodiesel production. There are three ways to reduce enzymatic biodiesel production cost. One is to reduce lipase production cost through development of a cheap and efficient method for lipase production; the second is to enhance lipase efficiency; and the last is the reuse of lipase. Among all, lipase reuse is the most feasible way. Immobilizing lipase on carriers, porous materials, is an effective method for lipase reuse. Various materials, such as fiber cloth, acrylic resin, silica gel, hydrotalcite, and macroporous or microporous materials, have been used as lipase carrier [1,58–60]. It has been indicated that the reused lipase could perform in terms of stability and activity as the initial one [58,61,62], which suggests that immobilization is a promising approach for lipase reuse.

3.1. Nanomaterial as biocatalyst carrier

Among all the carriers, nanomaterials have gained a great interest in the immobilization of lipase (Table 2). Nanomaterials are characterized with extensively large surface to volume ratio, which reveals that nanomaterials are capable of providing enormous surface area for lipase immobilization. In addition, extremely small pore sizes in nanomaterials enhance reactant diffusion rate to the active site of lipase because of that the diffusion rate is determined by the square of diffusion path accessing the active site (Eq. 2),

$$R_{df} \propto \frac{1}{D^2} \tag{2}$$

where R_{df} is the diffusion rate of reactant to active sites of enzymes, D is the diameter of diffusion path of reactants accessing to active sites of enzymes.

Thus the smaller D leads to greater R_{df} [63,64]. A high diffusion rate of reactants would accelerate the transesterification process. Furthermore, researchers have reported that nanomaterials used for lipase immobilization would retain or even enhance enzyme activity, selectivity, and stability [65–69].

It was studied the activity of the lipase immobilized onto carbon nanotubes (CNTs) in transesterification of ethyl butyrate and found that 97% activity of the lipase was retained as well as a high enantioselectivity (360) was shown [65]. It could probably be due to that the hydrophobic CNTs lead the active sites of lipase, which are located on the opposite direction with hydrophobic pocket of lipase, to an accessible orientation [70–72]. Moreover, it is predicated that terminal group of CNTs could be responsible for the retention of enzyme activity and stability [73]. Lipase encapsulated by polymer nanogel retained 80% activity after 2 h reaction, while free lipase retained less than 10% activity after 30 min reaction in transesterification of dextran and vinyl decanoate [67]. According to the result from molecule stimulation, the high activity retention could be due to the lipase structure perseveration under nanogel environment protection, while the high stability of the lipase was most probably attributed to firm lipase immobilization onto the network structure polymer gel. Kwon et al. [74] reported that lipase immobilized onto nanosized silica kept 93% activity yet free lipase only remained 40% activity after 7 months storage. In addition, it was reported that enhanced activity was achieved after immobilizing lipase onto surface modified zirconia nanoparticles and the activity retained as high as initially after reusing 8 times [66].

Immobilizing lipase onto nanomaterials showed rather encouraging results. Lipase reuse is accomplished by settling and centrifuging. The fact that usually nanomaterials have small particle sizes causes time consuming in settling and energy consuming in centrifuging. In order to overcome the problem, it was introduced the application of nanomagnetic materials in enzyme immobilization and found that the recovery of lipase could be easily and rapidly (within 1 min) completed by the addition of external magnetic fields [64,75]. It implies that immobilizing lipase onto nanomagnetic materials could be a strategy of enhancing the reusability of lipase.

In addition, it has been contested that the use of whole microbial cells containing lipase in transesterification was comparable with free lipase [76]. Utilization of whole cells instead of free lipase is more economical because of the prevention in lipase extraction, separation, and purification. Moreover, studies on

Table 2
Nanomaterial application in lipase immobilization.

Lipase source	Nanomaterials	Activity remaining (%)	Times of IRTA[a] of immobilized to free lipase	Times of TCR[b] of immobilized to free lipase	Reuse ability	Reference
Candida rugosa	Carbon nanotubes	97	2.2-14	4.44	—	[65]
Candida rugosa	Nanogel	85	—	7.67	—	[67]
Candida rugosa	Fe_3O_4 nanoparticles	80	110	20.5	4	[94]
Candida rugosa	ZrO_2	214	—	3.3	8	[66]
Candida rugosa	γ-Fe_3O_4 nanoparticles	< 100	—	—	—	[95]
Candida antarctica	Fe_3O_4 nanoparticles	200	—	—	4	[96]
Candida antarctica	Polystyrene nanoparticles	204	—	—	—	[97]
Pseudomonas cepacia	ZrO_2		—	3.6	-	[66]
Thermomyces lanuginosus	Nanosized silica	93	—	—	—	[74]
Thermomyces lanuginosa	Fe_3O_4 nanoparticles	70	—	1.05	4	[20]

[a] Initial rates of transesterification activity.
[b] Transesterification conversion rate.

Table 3
Nanocatalyst in transesterification.

Heterogeneous catalyst (nanosized)	Oil	Catalyst addition C/O[a] ratio (% w/w)	Reaction time (h)	Yield (%)	Reference
CaO	Poultry fat	0.6	12	99	[21]
CaO	Soybean oil	16	6	93.5	[98]
$Cs_2Mg(CO_3)_2$	Butter	—	3	100	[99]
KF/Al_2O_3	Soybean oil	3	8	99.84	[83]
$KF/CaO—Fe_3O_4$	Stillingia oil	4	3	95	[100]
KF/CaO—MgO	Rapeseed oil	3	3	95	[80]
KF/CaO	Tallow seed oil	—	—	96.8	[22]
$K_2O/\gamma\text{-}Al_2O_3$	Rapeseed oil	3	3	94	[101]
K_2CO_3/CaO	Soybean oil	3	1	99	[102]
Li—CaO	Karanja and jatropha oils	5	1	100	[103]
MgO	Soybean oil	2	17	99	[104]
MgO	Sunflower oil and rapeseed oil	1.5	6	90	[82]
MgO	Palm oil	0.5	4	51.3	[105]
$Zn_{1.2}H_{0.6}PW_{12}O_{40}$	Waste cooking oil	2.5	12	97.2	[85]

[a] Ratio presents catalyst to oil ratio.

whole cell immobilization utilization in transesterification have been reported [77–79]. It could be predicated that immobilized microbial whole cells containing lipase onto nanomaterials could be a cost-efficient method of biodiesel production.

Apart from biocatalyst (lipase), heterogeneous catalyst is found to be another efficient catalyst. Numerous heterogeneous catalysts, such as calcined Li–CaO, Mg–Al hydrotalcites, calcium oxides, magnesia-rich magnesium aluminate spinel, Mg/Zr, which are the most solid acid or base, have been investigated in biodiesel production [21,80,81]. Among all heterogeneous catalysts, nanocatalysts have become a competitive candidate because of the high catalytic efficiency and ease in separation from products (Table 3). Biodiesel production through nanocatalytic transesterification from various oils such as plant oils and waste oils have been reported [21,22,80,82,83]. It was revealed that to obtain similar reaction (transesterification) conversion, the amount of nanocatalyst required is only 30% of that of common catalysts, and additionally, the reaction is less affected by the moisture of the oil and not influenced by free fatty acid content [21,83–85]. Recently, nanocatalyst application in the transesterification of microalgae oil has also been reported. A novel bifunctional (acid–base) mesoporous silica nanomaterial catalyst was introduced and planned to use the catalyst in biodiesel synthesis from microalgae [19]. It was reported that lipid extracted from microalgae was converted into biodiesel using nanoparticle silica catalyst in pilot plant [16].

Nanotechnology application in biodiesel production could significantly impact on the current edible oil, microalgae lipid and biodiesel market. The growing price of edible oil leads to biodiesel production unaffordable. As discussed above, nanomaterial could improve lipid accumulation (increasing lipid content in biomass) in microalgae and hence increase lipid production from equal amount of microalgae biomass, which would reduce the production cost. In addition, implementing nanomaterial in lipid extraction without harm on microalgae prevents the cost which is demanded for recultivating microalgae after extraction in the current system. The utilization of nanomaterial as carrier for whole cell lipase or as catalyst in transesterification would provide high quality biodiesel and by-product glycerol due to the ease recovery of the solid nanomaterial compared to the generally used acid or base catalyst. Moreover, the downstream purification of biodiesel and glycerol is simplified which reduces the cost. On the other hand, the elimination of the usage of acid or base catalyst, which is not possible to be recovered and has to be neutralized with the addition of chemicals, could further reduce the biodiesel production cost. Overall, nanomaterial utilization could bring a revolution in biodiesel market.

4. Research need and future prospect

Heterotrophic microalgae have 10–20 folds higher growth rate than oleaginous crops and showed high lipid accumulation ability (up to 50% w/w of dry microalgae weight). The work on enhancing lipid accumulation through manipulating cultivation condition such as pH, temperature, carbon to nitrogen ratio, etc. should be performed as carbon and nitrogen sources were the only two factors have been reported till date.

The utilization of nanomaterial could enhance lipid production and transesterification, Specifically, in lipid production, the study on the fortification of nanomaterial in cultivation medium to stimulate lipid production/accumulation of heterotrophic microalgae should be conducted, and the utilization of nanomaterial instead of organic solvent which has safety and health issue, to achieve lipid extraction without killing the microalgae should be developed.

Immobilizing lipase onto nanomaterials has found to enhance lipid stability and reuse potential; however, the studies are mainly focusing on the utilization of nanoparticles and their separation from the products is difficult. Therefore, different types of nanomaterials such as the materials with nanosized pore or channels should be investigated for lipase immobilization; in order to accomplish easy separation of immobilized lipase from products, magnetic nanomaterials should be applied. Extracting lipase from microorganism is a costly process; therefore, whole cell lipase has been reported utilizing in transesterification. In order to complete its recovery from products, immobilizing the whole cell lipase onto nanomaterials should be investigated. The studies of nanomaterial application in biodiesel production are in lab-scale, for the practical utilization, pilot-scale study is required. As nanotechnology application in biodiesel production develops, biodiesel production using heterotrophic microalgae will be more sustainable than the current biodiesel production method.

5. Summary

Utilization of heterotrophic microalgae as feedstock is a promising way of biodiesel production. However, it has been hampered due to the costly lipid extraction process. Nanomaterials could efficiently achieve the extraction from microalgae cells, and appropriate selection on nanomaterials could even prevent harming microalgae. In addition, nanomaterial addition in the cultivation medium could enhance lipid accumulation of microalgae because it may affect the lipid metabolism.

Enhancing biodiesel production from heterotrophic microalgae through nanotechnology is still in the infant stage. Further research on the addition of nanomaterial such as nanosized silica and iron oxide to cultivation medium of heterotrophic microalgae should be investigated on the effect of lipid accumulation. The effort should be made on manipulation of the synthesis of nanomaterial containing function groups weakening/breaking cell

walls and dissolving lipid. Whole cell lipase immobilization on nanomaterials should be studied and optimized.

Acknowledgments

Sincere thanks are due to the Natural Sciences and Engineering Research Council of Canada (Grant A 4984, Canada Research Chair) for their financial support. The views and opinions expressed in this paper are those of the authors.

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